

CLAIMS

1. A method for identifying a protein present in a body fluid sample from a mammal, the method comprising the steps of:
 - introducing said sample to an affinity chromatography column functionalized with a ligand capable of selectively binding an abundant protein in the sample, wherein the affinity column produces an output with a reduced content of at least one abundant protein;
 - introducing said output having a reduced content of at least one abundant protein to a size exclusion column, wherein the size exclusion column separates proteins according to their size, and retaining the proteins of less than 20kD in one output fraction;
 - introducing said retained output fraction from said size exclusion column to at least one further chromatographic column, wherein said column(s) separates proteins according to an additional physical property and produces an effluent stream of eluted components; and
 - detecting the mass of a plurality of proteins present in said effluent stream, wherein the mass information is analyzed to identify a protein.
2. The method of claim 1, wherein said at least one further chromatographic column comprises: an ion exchange chromatography column, wherein the ion exchange column produces a first effluent stream of eluted components, and a reverse phase HPLC column, wherein the reverse phase HPLC column produces a second effluent stream of eluted components.
3. The method of claim 2, further comprising:
 - introducing eluted components from said second effluent stream to a second reverse phase HPLC column, wherein the second reverse phase HPLC column produces a third effluent stream of eluted components.
4. The method of any one of claims 1-3, wherein said detecting the mass of a plurality of polypeptides is accomplished using mass spectrometry.

5. A method for identifying the proteins present in a body fluid sample from a mammal, the method comprising the steps of:
 - introducing said sample in one or more runs to an affinity chromatography column functionalized with a ligand capable of selectively binding an abundant protein in the sample, wherein the affinity column produces an output having a reduced content of at least one abundant protein;
 - introducing a portion of said output having a reduced content of at least one abundant protein to a two-dimensional electrophoresis separation means;
 - introducing another portion of said output having a reduced content of at least one abundant protein to a size exclusion column, wherein the size exclusion column separates proteins according to their size, and retaining the proteins of less than 20kD in one output fraction;
 - introducing said retained output fraction from said size exclusion column to an ion exchange chromatography column, wherein the ion exchange column produces a first effluent stream of eluted components;
 - detecting a plurality of proteins separated using said two-dimensional electrophoresis means, wherein the two-dimensional electrophoresis information is analyzed to identify a protein; and
 - detecting the mass of a plurality of proteins present in said first effluent stream, wherein the mass information is analyzed to identify a protein.
6. The method of any one of claims 1 to 5, wherein said affinity chromatography column removes at least one abundant protein and at least 50% of the total protein mass of the body fluid sample.
7. The method of any one of claims 1 to 5, wherein said affinity chromatography column removes at least 80% of said abundant protein
8. The method of any one of claims 1 to 5, wherein said affinity chromatography column removes at least 90% of said abundant protein.
9. The method of any one of claims 1 to 5, wherein said affinity chromatography column removes at least 99% of said abundant protein.

10. The method of any one of claims 1 to 5, wherein at least 10 mL of body fluid is introduced to an affinity chromatography column.
11. The method of any one of claims 1 to 5, wherein the body fluid sample is derived from a single individual.
12. The method of claims 1 to 5, wherein the body fluid sample is derived from a plurality of individuals.
13. The method of claims 1 to 5, wherein the body fluid sample is a plasma sample.
14. The method of claim 13, wherein the sample is substantially undigested prior to said introduction to said size exclusion column.
15. The method of claim 13, wherein the sample is substantially undigested prior to said detection step.
16. The method of claims 1 to 5, wherein the sample is introduced in a plurality of runs.